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Pulmonary intravascular macrophages in deer

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Summary — Pulmonary intravascular macrophages (PIMs) have been found in the septal capillaries of deer lungs. Lung samples from adult deer were fixed in 2.5% glutaraldehyde, and then routinely processed for electron microscopy. The main features of the PIMs were the presence of tubular invaginations in the membrane (*micropinocytosis vermiformis*), phagosomes, and junctions with endothelial cells. A mean of 4.4 of these junctions was recorded per cell. They comprised segments ranging from 67 to 289 nm in length, where the plasma membranes were separated by spaces from 10 to 25 nm wide. In these areas the cytoplasm underlying the membranes showed evidence of increased electron density. When PIMs were compared with alveolar macrophages, it could be seen that although the PIMs were more numerous (more than twice), they were also smaller than the alveolar macrophages (47.625 versus 101.260 μm^2 respectively).

lung / macrophage / pulmonary intravascular macrophage / intercellular junction / deer

Résumé — **Macrophages intravasculaires pulmonaires chez le cerf.** *Des macrophages intravasculaires pulmonaires (MIPs) ont été mis en évidence dans les capillaires septaux du poumon de cerf. Des échantillons de poumon de cerfs adultes ont été fixés par le glutaraldéhyde à 2,5 % puis observés de façon routinière au microscope électronique. Les macrophages intravasculaires pulmonaires avaient pour caractéristiques principales la présence d'invaginations tubulaires de la membrane (micropinocytosis vermiformis), de phagosomes et de jonctions avec les cellules endothéliales. Les jonctions ont été observées à une moyenne de 4,4 par cellule. Elles étaient constituées de segments d'une longueur comprise entre 67 et 289 nm de large dont les membranes plasmatiques étaient séparées par des espaces ayant environ 10 à 25 nm de large. Dans ces zones le cytoplasme sous-jacent*

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aux membranes montrait une densité aux électrons accrue. En comparant les MIPs avec les macrophages alvéolaires, on a observé que, bien que les MIPs soient plus nombreux (plus de deux fois), ils avaient une taille plus petite que les macrophages alvéolaires (47,625 mm² vs 101,260 mm² respectivement).

poumon / macrophage / macrophage intravasculaire pulmonaire / jonction / cerf

INTRODUCTION

Pulmonary intravascular macrophages (PIMs) are mature cells of the mononuclear phagocyte system that form a resident population in the pulmonary capillaries of certain animals, mainly those belonging to the Artiodactyla order (Winkler, 1988; Staub, 1989; Warner and Brain, 1990; Chitko-McKown and Blecha, 1992; Longworth et al, 1994; Staub, 1994).

The existence of PIMs was first reported in 1974 (Rybicka et al, 1974), but they were not studied in any great depth until the late eighties, when several studies into their features and functional significance were made in the goat (Atwal et al, 1989), bovine (Rybicka et al, 1974; Warner and Brain, 1986; Atwal et al, 1989; Whiteley et al, 1991, 1992), sheep (Warner et al, 1986, 1987; Miyamoto et al, 1988; Warner et al, 1988), swine (Bertram, 1986; Morton and Bertram, 1988; Winkler and Cheville, 1987; Sierra et al, 1990; Carrasco et al, 1992) and horse (Atwal et al, 1992; Longworth et al, 1994). It is now recognized that PIMs are largely responsible for blood clearance in certain animal species, as greater titres of particles, endotoxins and bacteria have been found in the lung than in the liver or spleen of the species in which they are present (Crocker et al, 1981; Warner and Brain, 1986; Warner et al, 1986, 1987; Miyamoto et al, 1988; Warner et al, 1988; Winkler, 1988).

PIMs, however, do not appear to have the same function and development in all species. While they are abundant and highly functional in sheep, bovine and swine; in sheep and swine they are more numerous than alveolar macrophages (Warner et al,

1986; Sierra et al, 1990) and in swine they are roughly as numerous as Kupffer cells (Winkler, 1989; Carrasco et al, 1992). In species such as human, bat, rat, dog and rabbit they are not particularly numerous or active in blood clearance until failure of the hepatosplenic clearance systems occurs (Chitko-McKown and Blecha, 1992).

Results for the uptake of particles in the llama (Staub, 1989) and reindeer (Staub et al, 1992) suggest that PIMs are present in undomesticated animals, although morphological studies have yet to be performed.

The present study provides a morphological description of the existence of PIMs in the lungs of deer. The morphological characteristics are detailed, in particular the plaques involved in intercellular adhesion to endothelial cells in the septal capillaries.

MATERIALS AND METHODS

Lung samples taken from five, 4-year-old, adult deer (*Cervus elaphus*) were studied. The animals were all healthy and were not submitted to stress before sacrifice. No vascular disorders (congestion, hyperemia or edema) were observed in the lungs. Samples of each lobe were taken from all animals and were fixed by immersion in 2.5% glutaraldehyde buffered to pH 7.4 with 0.1 M phosphate buffer.

For transmission electron microscopy, tissue blocks of 1–2 mm³ were postfixed in 1% osmium tetroxide and stained in block with 0.5% uranyl acetate. Tissues dehydrated in graded alcohols and propylene oxide were embedded in epoxy resin (Epon 812; Fluka, Switzerland). Sections 50–70 nm thick were cut on an LKB Ultratome Nova ultramicrotome (LKB-Produkt AB, Bromma, Sweden), using a diamond knife (Diatome LTD, Bienne, Switzerland) and were

examined under a Philips CM-10 (Philips, Eindhoven, The Netherlands) electron microscope.

From each block, 0.5 μm thick sections were cut with glass knives, stained with toluidine blue, and scanned under a light microscope to ensure that only the parenchymal tissue was present.

To calculate the number of cells present, two alveolar tissue blocks from each lobe were selected for each animal. A cell count was performed in ten consecutive fields of 90 x 90 μm for each block. For the morphometrical study, a video-based, computer-linked, automatic image analyzer was used. To study cell area, ten photographs were taken of each block at 3 900 x. For the study of the junctions of PIM with the endothelial cells, ten photographs were taken of each block at 15 500 x. The images were recorded with a TV camera (model VK-C150ED; Hitachi, Japan) using a 50 mm lens, viewed on a monitor screen and measured by the morphometry program IMAGO (a standard morphometric software) developed by the Sistemas Inteligentes en Visión Artificial (SIVA) research team (University of Córdoba, Spain).

RESULTS

Inside the pulmonary capillaries there were flattened cells with an extensive area of apposition with the capillary endothelium (fig 1). In some areas, their membranes made contact with adjacent endothelial cells (figs 1 and 2). These junctions were comprised of short segments measuring 67–289 nm, and the membranes were separated by spaces 10–25 nm wide. In these areas, the membranes of both cell types were smooth and parallel, and the underlying cytoplasm revealed an increased electron density (fig 2). Submembrane linear densities ranged from 20 to 45 nm thick. A mean of 4.4 junctions per PIM was recorded.

These flattened cells displayed small cytoplasmic projections on the lumen surface, and the surface coat was preserved in the tubular invaginations of the membrane

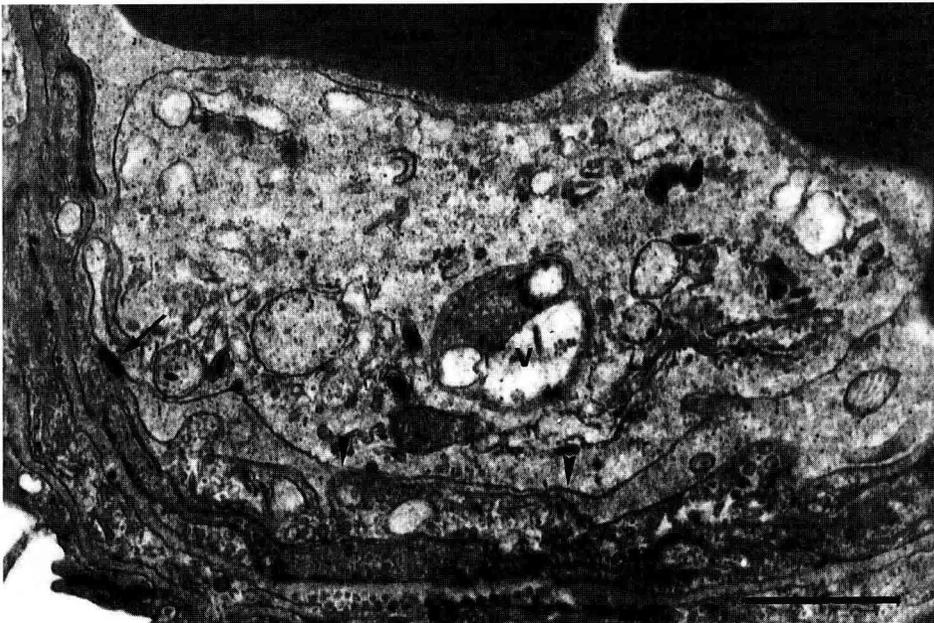


Fig 1. Pulmonary intravascular macrophage with a junction (arrow) and an extensive area of apposition (between arrowheads) with the capillary endothelium. The macrophage has a phagocytic vacuole (V). Bar: 1 μm .

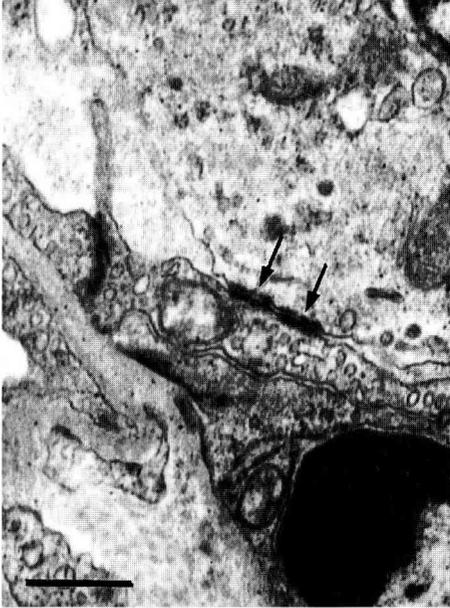


Fig 2. A higher magnification shows details of the junctions (arrows) between a pulmonary intravascular macrophage and the capillary endothelium. Bar: 1 μm .

(*micropinocytosis vermiformis*) (fig 3). Moderate numbers of mitochondria, some free ribosomes, and a few lipid globules were present in the cytoplasm, together with small lysosomes and the occasional appearance of phagosomes (fig 1). This combination of characteristics allowed these cells to be identified as PIMs. Their area was approximately $47.625 \mu\text{m}^2$, the cytoplasmic to nuclear volume ratio was 2.7, and there was a mean of 1.525 cells per field of $40 \times 40 \mu\text{m}$.

The deer lung samples also contained alveolar macrophages, with a mean of 0.625 cells per $40 \times 40 \mu\text{m}$ field. They were large, with an area of around $101.260 \mu\text{m}^2$. They had an uneven profile, and abundant surface projections. The cells had a cytoplasmic to nuclear volume ratio of 4.1, and the cytoplasm clearly revealed the presence of lysosomes and phagosomes, of which the latter contained surfactant material (fig 4).

DISCUSSION

The cells found in the septal capillary lumen were identified as PIMs, based on the following ultrastructural characteristics: i) cell junction with capillary epithelium; ii) tubular structure of *micropinocytosis vermiformis*; and iii) phagosomes. These make them different from other types of cells found in the lung (Bertram, 1986; Warner and Brain, 1986; Warner et al, 1986, 1987; Winkler and Chevillat, 1987; Morton and Bertram, 1988; Warner et al, 1988; Winkler, 1988; Atwal et al, 1992; Chitko-McKown and Blecha, 1992; Longworth et al, 1994).

While endothelial junctions are one of the main features of PIMs, and indeed their presence has been reported for all of the species in which these cells have been identified, an in-depth study of their characteristics has only been carried out in swine (Bertram, 1986; Morton and Bertram, 1988). The junctions found in deer PIMs present features similar to those of swine and sheep, with a length of under $0.5 \mu\text{m}$ and membrane separation of around 15 nm (Bertram, 1986; Warner et al, 1986; Morton and Bertram, 1988; Winkler, 1988). Sub-plasma membrane thickening was slightly less than that reported for swine (Bertram, 1986; Morton and Bertram, 1988) and the number of unions was lower with a mean of 4.4 per PIM compared to 5.8 reported for swine (Bertram, 1986). The junctions between PIMs and endothelial cells have been referred to as intercellular adhesion plaques (Bertram, 1986; Morton and Bertram, 1988), and are distinguishable from the subplasmalemma linear densities present in interstitial macrophages and from the epithelioid cells found in some lung diseases, since these cells have lengths ranging from 0.28 to $2.6 \mu\text{m}$ and have an intermembrane space of $50\text{--}80 \text{ nm}$ (Kawanami et al, 1980).

The presence of *micropinocytosis vermiformis* in the cells would indicate that these cells are well differentiated and are

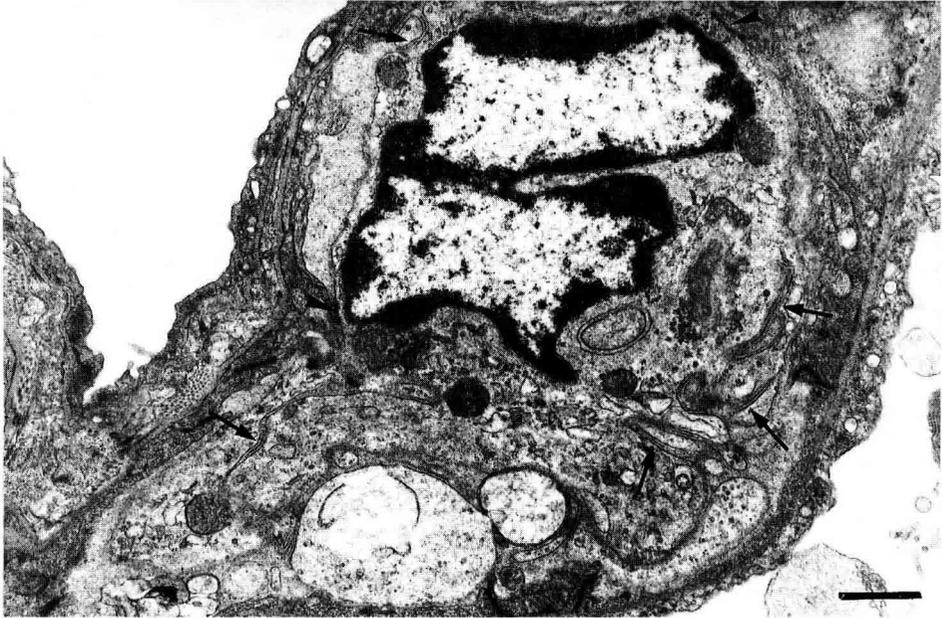


Fig 3. Pulmonary intravascular macrophage with two junctions with the capillary endothelium (arrowheads) and numerous *micropinocytosis vermiformis* (arrows). Bar: 1 μm .

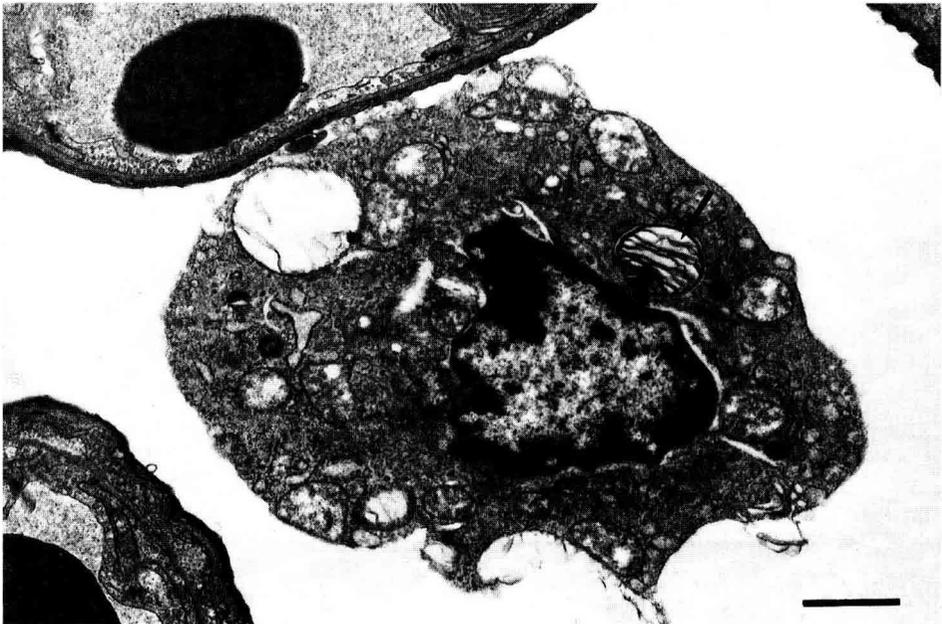


Fig 4. Alveolar macrophage within the alveolar space. Engulfed surfactant can be seen within phagocytic vacuole (arrow). Bar: 1 μm .

not simply attached monocytes (Warner and Brain, 1990). These structures are also prominent in the PIM cytoplasm of the cat, bovine, sheep, goat, horse and swine (Schneeberger-Keely and Bruger, 1970; Rybicka et al, 1974; Bertram, 1986; Warner et al, 1986; Winkler, 1988; Atwal et al, 1992; Longworth et al, 1994), and have a corresponding invagination of the glycocalyx (Winkler, 1988; Warner and Brain, 1990).

In deer, we found no differences between the number of PIMs in the different pulmonary lobes, in agreement with the findings for sheep reported by Warner et al (1986), who concluded that a sample taken from any lobe would be representative of the lung.

Although few studies have compared PIMs with alveolar macrophages, it was estimated in sheep (Warner et al, 1986) and swine (Sierra et al, 1990) that the number of PIMs is greater than that of alveolar macrophages. The results of the present study demonstrated that there were twice as many in deer as well. In deer, the alveolar macrophages were also larger than PIMs, in agreement with the reports for swine (Morton and Bertram, 1988; Chitko-McKown et al, 1991).

The existence of PIMs should be beneficial to the animal, since they actively participate in the blood clearance of senescent erythrocytes, cell debris, fibrin, bacteria and endotoxins (Bertram, 1986; Winkler, 1988; Warner et al, 1987, 1988; Warner and Brain, 1990; Whiteley et al, 1991, 1992). The uptake of bacteria or toxins by PIMs and their subsequent activation have been associated with pulmonary pathological changes, characterized by neutrophil accumulation in capillaries, pulmonary endothelial injury, increased microvascular permeability with a consequent interstitial and alveolar edema (Warner et al, 1987, 1988; Whiteley et al, 1991, 1992) and a microvascular thrombosis (Warner et al, 1988; Whiteley et al, 1991, 1992). Pathological changes that have been

related to the possible release of interleukin-1, tumor necrosis factor, platelet activating factor, leukotrienes, oxygen metabolites (Warner et al, 1988; Warner and Brain, 1990; Whiteley et al, 1991, 1992) and arachidonic acid metabolites (Miyamoto et al, 1988) by activated PIMs, could be due to the capacity of this kind of cell to produce them (Chitko-McKown et al, 1991; Chitko-McKown and Blecha, 1992).

The species in which PIMs have been found are not related by a common lineage with respect to overall anatomy, taxonomic classification, or pulmonary microanatomy. Why these particular species have developed resident macrophages in their pulmonary capillaries remains unclear (Warner and Brain, 1990; Staub, 1994). The presence of the PIMs, however, is related to a high retention of foreign particles in the pulmonary circulation and a greater sensitivity to endotoxin. It is for these reasons that deer may have a similar behaviour to sheep, goat or bovine in their development of respiratory diseases caused by Gram-negative bacteria or those that develop as a consequence of stress, such as pleuropneumonia or influenza virus infection.

The present study has demonstrated the existence of pulmonary intravascular macrophages in deer. These cells form a resident population in the pulmonary circulation and are attached to endothelial cells by junctions similar to those reported for PIMs in swine. The role played by PIMs in deer is unknown, although their existence is strongly linked to the pathophysiological mechanisms of lung injury and disease.

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